Chapter 2

Low essential fatty acid and B-vitamin status in a subgroup of patients with schizophrenia and its response to dietary supplementation

Ramses F.J. Kemperman [1,2]
Marieke Veurink [1]
Tineke van der Wal [3]
Henderikus Knegtering [3]
Richard Bruggeman [3]
M. Rebecca. Fokkema [1]
Ido P. Kema [1]
Jaap Korf [3]
Frits A.J. Muskiet [1]

[1] Pathology and Laboratory Medicine, University Medical Center Groningen,
[2] University Center for Pharmacy, Analytical Biochemistry, University of Groningen,
[3] Psychiatry, University Medical Center Groningen;
Groningen, The Netherlands.

Kemperman RFJ, Veurink M, van der Wal T, Knegtering H, Bruggeman R, Fokkema MR, Kema IP, Korf J, Muskiet FAJ. Low essential fatty acid and B-vitamin status in a subgroup of patients with schizophrenia and its response to dietary supplementation. Prostaglandins Leukot Essent Fatty Acids. 2006 Feb;74(2):75-85. Epub 2005 Dec 27.

Abstract

We assessed essential fatty acid (EFA) and B-vitamin status, together with their determinants, in 61 patients with schizophrenia and established whether those with poor status responded biochemically to the appropriate dietary supplements. As a group, the patients had high erythrocyte saturated fatty acids (FA), monounsaturated FA and low polyunsaturated FA of the ω3 and ω6 series. Patients reporting not to take vitamin supplements had low vitamin B_{12} and high homocysteine. Homocysteine variance proved best explained by folate in both the total group and male patients, and by vitamins B_{12} and B_6 in females. Alcohol consumption and duration of illness are risk factors for low polyunsaturated FA status (<P2.5 of reference range), while male gender and absence of fish consumption predict hyperhomocysteinemia (>P97.5 of reference range). Two patients exhibited biochemical EFA deficiency and 7 showed biochemical signs of ω3/docosahexaenoic acid (DHA) marginality. Four patients exhibited moderate hyperhomocysteinemia with plasma values ranging from 57.5-74.8 μmol/L. None of the 5 patients with either moderate hyperhomocysteinemia, biochemical EFA deficiency, or both, was predicted by their clinicians to have a poor diet. That diet was nevertheless at the basis of these abnormalities became confirmed after supplementing 4 of them with B-vitamins and with soybean and fish oils. We conclude that a subgroup of patients with schizophrenia has biochemical EFA deficiency, ω3/DHA marginality, moderate hyperhomocysteinemia, or combinations. Correction seems indicated in view of the possible relation of poor EFA and B-vitamin status with their psychiatric symptoms, but notably to reduce their high risk of cardiovascular disease.

1. Introduction

Patients with schizophrenia have frequently been shown to have a low status of essential fatty acids (EFA) of both the ω3 and ω6 series. Low erythrocyte (RBC) contents of linoleic acid (LA; 18:2ω6) and the long chain polyunsaturated fatty acids (LCP) arachidonic acid (AA; 20:4ω6) and docosahexaenoic acid (DHA; 22:6ω3) are the most consistent findings [1]. Some results of former studies may have been confounded by LCP losses in the pre-analytical phase, which might be due to the higher sensitivity of the LCP in the RBC of patients with schizophrenia to oxidative stress *in vitro* [2]. Other possible causes for low LCP status in schizophrenia might relate to a reduced rate of LCP incorporation into phospholipids and increased phospholipase-catalyzed loss (the so called 'membrane phospholipid hypothesis' [3]), the interaction with gender and smoking, or low dietary intake [4;5]. Low dietary intake of LCP [6] and low RBC LCP status [7;8], notably AA and DHA, are associated with an increase of psychiatric symptoms that may be at the basis of the well known cross-cultural differences in schizophrenia severity [9;10]. A meta-analysis of 2003 concluded that the use of ω3LCP in schizophrenia is still experimental and that the outcome of large, well designed, studies has to be awaited [11]. At present six out of 7 trials with (add-on) eicosapentaenoic acid (EPA; 20:5ω3) supplements yielded positive results on psychiatric end points [4;12;13].

Patients with schizophrenia also have low functional B-vitamin status which becomes noticeable from above-normal levels of plasma homocysteine [14-18]. Increased homocysteine levels in schizophrenic patients have, however, not consistently been found [19-21]. Homocysteine is a cytotoxic amino acid that is likely to be involved in both neurodegenerative disorders, like Alzheimer's disease [22], and psychiatric disorders, including schizophrenia [23]. The major determinants of increased plasma homocysteine in the general population are low serum folate, vitamin B_{12} and betaine [24-26], which share functions in one-carbon metabolism [27]. Folate status in schizophrenic patients correlates inversely with negative symptoms [19] and in one study supplementation with methylfolate was found to enhance social and clinical recovery [28].

Life expectancy of patients with schizophrenia is 20% shorter compared with the general population. The excess mortality is for 60% attributable to physical illness (circulatory, respiratory, digestive and genitourinary disease) with the remainder on account of suicide (28%) and accidents (12%) [29]. Some newer atypical antipsychotic drugs may have side effects such as weight gain, elevation of serum triglycerides and increased risk of diabetes mellitus type 2. All of these constitute risk of cardiovascular disease in a population segment with little exercise,

poor diet, almost universal smoking, and unhealthy lifestyle in general [13;29]. In other words, patients with psychiatric diseases, especially patients with schizophrenia, may benefit from good nutrition, and not merely with the aim of ameliorating psychiatric end points. Also mild hyperhomocysteinemia [30;31] and low ω3LCP status [32-34] are risk factors for cardiovascular disease events and death. No matter the origin and whether these are features of all patients, both mild hyperhomocysteinemia and low ω3LCP status are correctable by supplementation of folic acid (or a folic acid, vitamin B_{12} and vitamin B_6 combination) and fish oil, which calls for patient-individual dietary counseling and dietary supportive care, if necessary.

Using a cross-sectional study design we determined the frequency of low EFA and B-vitamin status in a representative group of patients with a schizophrenia spectrum disorder. We were also interested to see whether low status of EFA and B-vitamin in patients is predictable by their physicians. Finally, we examined whether those with low statuses improved biochemically after short term supplementation of soybean and fish oils and B-vitamins, including folic acid.

2. Patients and Methods

2.1. Patients, samples and analyses

One hundred patients, classified as having schizophrenia according to the DSM-IV TR [35], were randomly selected from the files of the department of Psychiatry of the University Medical Center of Groningen in The Netherlands. The selected patients were asked to participate through invitation by mail, telephone or during therapeutic sessions with two of us (HK, RB). There were no other inclusion or exclusion criteria. Sixty-one (61%) patients agreed to participate. The study was performed in the summer of 2003. The protocol was in agreement with local ethical standards and the Helsinki declaration of 1964, as revised in 2002.

The participants were physically examined and their anthropometrical data were recorded. Information regarding smoking, alcohol consumption, drug abuse, use of nutritional supplements and fish consumption was obtained with the aid of an assisted questionnaire. Ages at onset of the illness, comorbidity and contemporary medication were taken from the hospital records. Non-fasting blood samples were obtained by venepuncture between 10.00 and 16.00 h for analyses in whole blood, serum, EDTA-plasma and isolated RBC. We analyzed routine clinical chemical and hematological indices in serum and EDTA-blood by standard clinical chemical methods. Serum folate and vitamin B_{12} (cyanocobalamin) were analyzed with a fluoroimmunoassay (Autodelfia, Wallac Oy, Germany), plasma homocysteine with an immunochemical method (ImX, Abbott Laboratories,

Illinois, USA), whole blood vitamin B_6 (pyridoxine) by HPLC [36] and the RBC fatty acid composition (expressed in mol%) by capillary gas chromatography [37]. Addition of the RBC to the antioxidant-fortified transmethylation mixture, following the immediate isolation of RBC by washing, ensured the stability of unsaturated fatty acids in the preanalytical phase [37]. The typical day-to-day coefficients of variation range from 1.1-17.6%, dependent on the abundance of the analyte expressed in mol% [38].

2.2. Supplementation of patients with poor B-vitamin and EFA status

Patients with isolated biochemical EFA deficiency were supplemented with a combination of soybean and fish oils and those with isolated moderate hyperhomocysteinemia were supplemented with B vitamins. For those with combined biochemical EFA deficiency and moderate hyperhomocysteinemia we chose to supplement first with EFA and second with B vitamins. No supplements were to be taken during the day prior to sampling. The patients were asked to continue supplementation beyond the twelfth week until they had their blood samples taken.

Patients with biochemical EFA deficiency (i.e. RBC 20:3ω9>0.46 mol% [38]) were supplemented for 12 weeks with purified fish oil capsules (Triomar; Pronova Biocare, Norway) corresponding with daily dosages of 310 mg EPA and 200 mg DHA. In this period they also received 15 mL soybean oil per day (generic brand from local grocer; Albert Heijn, The Netherlands) corresponding with daily dosages of 1 g ALA and 7 g LA. Soybean oil was added to the hot meal. Folic acid (800 μg) was added to this regimen from weeks 4 to 6 and the B-vitamin combination (800 μg folic acid, 8 mg vitamin B_6, 4 μg vitamin B_{12}) was provided from weeks 6 to 12. Generic brand folic acid and B-vitamin supplements were purchased from a local drugstore (Etos, The Netherlands). Blood samples for analyses were taken at 0, 4, 6 and 12 weeks.

Patients with moderate hyperhomocysteinemia (i.e. homocysteine levels ranging from 31-100 μmol/L [31;39]) were selected for supplementation with 800 μg folic acid per day during 2 weeks. They were subsequently supplemented for 10 weeks with an additional B-vitamin preparation, which resulted in a total daily intake of 800 μg folic acid, 8 mg vitamin B_6 and 4 μg vitamin B_{12}. From 4 to 12 weeks the patients also received the same daily dosages of fish and soybean oils (310 mg EPA, 200 mg DHA and 15 mL soybean oil). Blood samples for the analyses of B-vitamin status parameters and RBC fatty acids were taken at 0, 2, 4 and 12 weeks.

2.3. Data evaluation and statistics

Patient characteristics (i.e. BMI, smoking and use of alcohol) were compared with age and sex matched data of the Dutch general population, as derived from the 2003 Dutch National Food Consumption Survey [40]. Subgroups were defined according to medication type i.e. clozapine, single atypical antipsychotic, multiple antipsychotics and classical antipsychotics. Antipsychotic medication dose was expressed as defined daily dose [41] and chlorpromazine (CPZ) equivalents [42]. Fish fat intake was expressed as 'Q', which equals the product of the number of days of fish consumption per week and a measure for the average fatness of the fish consumed. The average fatness ranged from lean (0.5-5% fat/g flesh) to oily (>5% fat/g flesh), giving rise to a measure of fatness ranging from 1 to 4. Using this index of fish fat intake (Q), the recommended intake of at least two servings of medium-oily fish per week [43] would translate into a Q of 4.

Clinical chemical data were evaluated with the use of reference values as applied in our laboratory. For RBC fatty acids we used reference values (i.e. 2.5 and 97.5 percentiles of healthy omnivorous controls) as previously reported [38]. These cut-off values are independent from the age of 0.2 years and apply for both sexes. RBC 20:3ω9 above 0.46 mol% was defined as biochemical EFA deficiency; an RBC 22:5ω6/AA ratio above 0.068 mol/mol was considered to reflect isolated ω3-deficiency; an RBC 22:5ω6/DHA ratio above 0.22 mol/mol but equal or below 0.48 mol/mol was defined as ω3/DHA marginality; and an RBC 22:5ω6/DHA ratio above 0.48 mol/mol was indicative for ω3/DHA deficiency [38]. For homocysteine we employed the 14.6 μmol/L upper limit of the reference range (i.e. P97.5) as derived from our previous study of healthy controls [44]. In addition we used a cut-off value (P97.5) of 9.3 μmol/L, as derived from data of apparently healthy subjects following supplementation with folic acid and the vitamins B_{12} and B_6 ('vitamin-optimized cut-off value'; [44]). These cut-off values apply for both sexes, but may provide a somewhat conservative estimate for premenopausal female patients. Numbers of patients with scores below and above the local reference values were calculated and expressed as percentages of the total. Stratification for age was employed to investigate the age-dependent increase of homocysteine in young male patients [14;15].

Statistical analyses were performed with the Statistical Product and Service Solutions package version 11.5 (SPSS Inc., Chicago). Differences in characteristics between patients and the Dutch general population were tested by Chi-square tests. Differences between patient and control data were tested at $p<0.05$ using either the Students' t or Mann-Whitney U tests, dependent on the normality of the distributions. P-values were corrected for relevant confounders (i.e. age and gender) using ANOVA or logistic regression, if applicable. Comparison of multiple

subgroups (e.g. based on medication or DSM-IV TR classification) was performed with ANOVA (parametric) after Bonferroni correction or with the Kruskall-Wallis (non-parametric) tests. Bivariate correlations were examined by calculating either the Pearson (linear) or Spearman (non-linear) coefficients. Outcomes were considered significant at $p<0.05$. RBC fatty acids were examined for correlation with B-vitamin status parameters to explore general patterns in nutrition. Predictors of low PUFA (<P2.5 of reference range) and increased homocysteine (>P97.5 of the reference range) in the whole group were examined by multinominal logistic regression in which we tested a selected number of indices that in bivariate comparisons proved most significantly related to PUFA and homocysteine, respectively. Multivariate linear regression was used to explain variance in RBC 20:3ω9 and plasma homocysteine. For this we tested the parameters that exhibited the most significant bivariate correlations.

3. Results

3.1. Patients

Table 1 depicts the patient characteristics. The percentages female and male patients with overweight (BMI>25 kg/m^2) were similar to the age- and gender-matched data of the Dutch general adult population. The percentage obesity (BMI>30 kg/m^2) was higher in female patients (30 vs. 11%; $p<0.001$), but not in male patients. The percentages smoking (55 vs. 30.8%; $p<0.001$), and heavy smoking (31 vs. 8.0%; $p<0.01$) were higher, which was notably on account of heavy smoking in female patients (39 vs. 8%; $p<0.0001$). The percentage patients consuming alcohol was lower (65 vs. 81.7%; $p<0.005$), although the percentage male patients reporting heavy drinking almost reached significance (27 vs. 17.0%; $p= 0.053$). The percentage patients reaching the recommended fish intake ($Q\geq4$) was 21%, while 10% reported not to consume fish at all. Smoking, alcohol and fish consumption did not differ significantly between sexes. The patients reporting intake of vitamins and/or fish oil supplements had better B-vitamin and LCPω3 status, respectively (data not shown). Patients reporting gastrointestinal disturbances (24%) did not differ in their characteristics, RBC fatty acid composition and B-vitamin status parameters, compared with those not having such complaints (not shown).

Table 1. *Patient characteristics.*

Gender *(M / F)*	37 | 24		
Age *(years)*	31.5 (9.2)		
BMI *(kg/m²)* [a]	25.5 (4.1)		
BMI *(<18.5 / 18.5-25 / 25-30 / >30)* [a]	0 | 26 | 18 | 8		
Hospitalized | Community dwelling	14 | 47		
Age at onset of illness *(years)* [b]	23 (16-45)		
Duration of illness *(years)* [b]	5 (1-20)		
Diagnosis according to DSM-IV			
295.3: paranoid type	21/61 (34%)		
295.4: schizophreniform disorder	9/61 (15%)		
298.9: psychotic disorder NOS	8/61 (13%)		
295.9: undifferentiated type	7/61 (12%)		
other	16/61 (26%)		
Medication		**Dose (mg/day)**	**Defined Daily Dose** [c]
olanzapine (N05AH03)	19/61 (31%)	11.8 (5-20)	1.18 (0.5-2)
risperidon (N05AX08)	19/61 (31%)	3.0 (1-8)	0.6 (0.2-1.6)
clozapine (N05AH02)	9/61 (15%)	363 (200-500)	1.21 (0.67-1.67)
other	17/61 (28%)		
no medication	2/61 (3%)		
Average dose		280.5 (78.3)[d]	0.94 (0.26)
	Yes (fraction; %)	**Median (range)**	
Diabetes Mellitus [e]	1/53 (2%)		
Smoking [f]	30/56 (55%)		
Cigarettes/day		20 (1-40)	
heavy smoking [g]	17/56 (30%)		
cigarettes/day in heavy smokers		20 (20-45)	
Recent use of cannabis [b]	12/55 (22%)		
Alcohol [f]	36/56 (64%)		
units/day [h]		0.7 (0.1-23)	
heavy drinking [i]	10/56 (18%)		
units/day in heavy drinking		3.9 (3-23)	
GI disturbances [j]	14/58 (24%)		

Table 1. *Patient characteristics* **(continued).**

	Yes (fraction; %)	Median (range)
Fish intake [j]	52/58 (90%)	
Q [k]		1 (0.04-21)
Q≥4 of those eating fish	11/52 (21%)	
Q of those with Q≥4		8 (4-21)
Use of supplements [e]	5/53 (9%) [l]	

Data are for 61 patients unless otherwise indicated. Data represent numbers, fractions (%), means (SD), or medians (range). [a] n=52, BMI of the patient with diabetes mellitus was 22.3 kg/m²; [b] n=55; [c] defined daily dose calculated with [41]; [d] chlorpromazine equivalents calculated with [41;42]; [e] n=53; [f] n=56; [g] ≥20 cigarettes per day; [h] 1 unit equals 20 mL of pure ethanol; [i] ≥3 alcohol units per day; [j] n=58; [k] Q is an index for fish fat intake, that equals the product of the number of days of fish consumption per week and an arbitrary measure for the average fatness of the fish consumed; [l] vitamins n=4, fish-oil supplements n=2.

3.2. Erythrocyte fatty acids

Table 2 shows the RBC fatty acid composition of patients and controls together with the percentage patients with values below and above the respective reference values. Between (sub)groups comparisons were done with patients reporting no intake of fish-oil supplements. Type or doses of antipsychotic medication were not significantly related to the RBC fatty acid composition. Most remarkable was the higher RBC saturated fatty acids (SAFA) content of the patients ($p<0.001$), with 19 (31%) patients exhibiting levels above the P97.5. Higher SAFA was notably attributable to higher 16:0 ($p<0.001$) and 12 (20%) patients had levels above P97.5. The percentage monounsaturated fatty acids (MUFA) ($p<0.001$), notably 18:1ω9 ($p<0.001$), was also higher, with 15 (25%) and 33 (54%) patients having contents above the P97.5, respectively. Higher RBC SAFA and MUFA coincided with lower polyunsaturated fatty acids (PUFA) ($p<0.001$), with 25 (41%) patients having contents below the P2.5. The lower PUFA status was attributable to different combinations of lower status of ω3 ($p<0.001$; 13% below P2.5), ω6 ($p<0.001$; 13% below P2.5), alpha-linolenic acid (ALA; 18:3ω3) ($p<0.001$; 36% below P2.5), DHA ($p<0.001$; 13% below P2.5), AA ($p<0.05$; 11% below P2.5) and 22:4ω6 ($p<0.001$; 11% below P2.5). Biochemical EFA deficiency (i.e. RBC 20:3ω9>0.46 mol%) was found in one male and one female patient, both 53 years of age of which one reported to not eat fish at all, whereas no such data were available from the other. Isolated biochemical ω3-deficiency, as indicated by increased RBC 22:5ω6/AA ratio, was not observed in our study group.

Biochemical ω3/DHA marginality, as derived from a 22:5ω6/DHA ratio >0.22 but ≤0.48 mol/mol, was observed in 9 (15%) patients, including the 2 with biochemical EFA. We did not observe biochemical ω3/DHA deficiency. Of the remaining 7 with biochemical ω3/DHA marginality 4 reported not to eat fish and no such data were available for one.

Table 2. ***Erythrocyte fatty acid composition of patients with schizophrenia.***

(right page)

Data are for 61 patients unless otherwise indicated. Data are presented in mol%. Reference values represent medians (P2.5-P97.5) for controls (n=69). Data for patients represent medians (range). The reference values (i.e. 2.5 and 97.5 percentiles) of the controls were used as cut-off values to evaluate individual patients. The outcomes are expressed as the number (%) of patients with RBC fatty acid content either below the P2.5 (n<P2.5) or above the P97.5 (n>P97.5). P<0.05 was considered significant for between group analyses. Biochemical essential fatty acid deficiency was defined as an RBC 20:3ω9 above 0.46 mol%. Biochemical isolated ω3 deficiency was defined as an RBC 22:5ω6/AA above 0.068 mol/mol. Omega-3/DHA marginality was defined as an RBC 22:5ω6/DHA above 0.22 mol/mol but equal or below 0.48 mol/mol, while ω3/DHA deficiency was indicated by an RBC 22:5ω6/DHA above 0.48 mol/mol [38]. ALA, alpha-linolenic (18:3ω3); EPA, eicosapentaenoic acid (20:5ω3); DHA, docosahexaenoic acid (22:6ω3); LCP, long chain polyunsaturated fatty acid; LA, linoleic acid (18:2ω6); AA, arachidonic acid (20:4ω6); SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. [a] Significance (p) for all patients (n=61) vs. controls; [b] Significance (p) for patients reporting no intake of fish-oil supplements (n=59) vs. controls; [c] Patient (n=1) reporting intake of fish-oil supplements is included; [d] Patients (n=2) reporting intake of fish-oil supplements are included.

	Reference values		*Patients*		*Patients*		*Patients vs. controls*	
	median	(P2.5 - P97.5)	median	(range)	n <P2.5	n>P97.5	p[a]	p[b]
14:0	0.43	(0.30 - 0.54)	0.46	(0.26 - 0.76)	2 (3)	11 (18)	0.103	0.146
16:0	23.77	(22.50 - 25.30)	24.35	(22.91 - 26.83)	0 (0)	12 (20)[c]	<0.001	<0.001
18:0	16.41	(15.18 - 17.78)	16.8	(14.53 - 19.41)	1 (2)	5 (8)[c]	0.001	<0.001
20:0	0.44	(0.36 - 0.53)	0.42	(0.31 - 0.67)	8 (13)	1 (2)	0.419	0.394
22:0	1.85	(1.50 - 2.17)	1.69	(1.32 - 2.15)	4 (7)[c]	0 (0)	<0.001	<0.001
24:0	4.62	(3.88 - 5.19)	4.44	(3.52 - 5.27)	4 (7)	1 (2)	0.129	0.169
26:0	0.23	(0.17 - 0.30)	0.23	(0.16 - 0.33)	3 (5)	2 (3)[c]	0.342	0.436
ALA	0.18	(0.13 - 0.28)	0.14	(0.08 - 0.34)	22 (36)	1 (2)	<0.001	<0.001
EPA	0.44	(0.23 - 0.93)	0.5	(0.16 - 1.98)	2 (3)	2 (3)[d]	0.223	0.501
22:5ω3	1.85	(1.40 - 2.46)	1.87	(1.24 - 2.63)	2 (3)	3 (5)[d]	0.878	0.587
DHA	3.75	(2.29 - 5.45)	3.13	(1.34 - 5.91)	8 (13)	1 (2)[c]	<0.001	<0.001
LCPω3	6.17	(4.39 - 8.28)	5.45	(3.35 - 10.36)	7 (11)	2 (3)[d]	<0.05	<0.005
ω3	6.38	(4.59 - 8.44)	5.69	(3.44 - 10.53)	8 (13)	2 (3)[d]	<0.05	<0.005
LA	10.28	(8.26 - 13.03)	9.79	(8.17 - 12.70)	1 (2)	0 (0)	0.051	0.074
20:2ω6	0.26	(0.18 - 0.42)	0.23	(0.15 - 0.41)	4 (7)	0 (0)	<0.005	<0.05
20:3ω6	1.57	(1.23 - 2.14)	1.78	(1.10 - 3.05)	4 (7)	12 (20)	<0.05	<0.05
AA	13.87	(12.15 - 15.91)	13.42	(9.96 - 16.34)	7 (11)[c]	1 (2)	<0.05	<0.01
22:4ω6	2.77	(1.99 - 3.53)	2.49	(1.46 - 3.28)	7 (11)[c]	0 (0)	<0.001	<0.001
22:5ω6	0.50	(0.35 - 0.70)	0.47	(0.29 - 0.76)	8 (13)[d]	2 (3)	<0.05	0.097
LCPω6	18.73	(16.42 - 21.42)	18.72	(13.79 - 20.29)	5 (8)[c]	0 (0)	0.057	0.136
ω6	29.31	(26.48 - 31.87)	28.62	(23.51 - 31.42)	8 (13)[d]	0 (0)	<0.001	<0.005
18:1ω7	1.76	(1.36 - 2.23)	1.27	(0.93 - 2.65)	42 (69)[c]	1 (2)	<0.001	<0.001
ω7	1.84	(1.40 - 2.31)	1.27	(0.93 - 2.65)	42 (69)[c]	1 (2)	<0.001	<0.001
18:1ω9	10.76	(9.16 - 11.48)	11.61	(9.63 -13.50)	0 (0)	33 (54)[c]	<0.001	<0.001
20:1ω9	0.22	(0.14 - 0.31)	0.22	(0.13 - 0.30)	2 (3)	0 (0)	0.451	0.489
20:3ω9	0.25	(0.13 - 0.42)	0.17	(0.09 - 0.67)	13 (21)	2 (3)	<0.001	<0.001
24:1ω9	3.37	(2.65 - 4.29)	3.75	(2.47 - 4.71)	1 (2)	5 (8)	<0.001	<0.001
ω9	14.48	(13.05 - 15.93)	15.78	(13.72 - 18.52)	0 (0)	27 (44)[c]	<0.001	<0.001
SAFA	47.76	(46.68 - 48.88)	48.57	(46.49 - 53.82)	1 (2)	19 (31)[c]	<0.001	<0.001
MUFA	16.1	(14.44 - 17.54)	16.89	(14.90 - 19.50)	0 (0)	15 (25)[c]	<0.001	<0.001
PUFA	36.13	(34.39 - 37.72)	34.46	(28.62 - 37.24)	25 (41)[c]	0 (0)	<0.001	<0.001
LCPω3+LCPω6	25.19	(23.16 - 27.57)	24.18	(19.57 - 26.86)	12 (20)	0 (0)	<0.001	<0.001
LCPω3/LCPω6	0.32	(0.22 - 0.48)	0.29	(0.18 - 0.75)	4 (7)	3 (5)[d]	0.325	0.062
PUFA/SAFA	0.76	(0.72 - 0.80)	0.71	(0.53 - 0.80)	36 (60)[d]	0 (0)	<0.001	<0.001
ω3+ω6	35.79	(33.83 - 37.74)	34.31	(27.94 - 37.11)	23 (38)	0 (0)	<0.001	<0.001
ω3/ω6	0.21	(0.15 - 0.31)	0.20	(0.11 - 0.45)	6 (10)	2 (3)[d]	0.399	0.096
20:3ω9/20:4ω6	0.02	(0.01 - 0.03)	0.01	(0.01 - 0.06)	11 (18)	3 (5)	0.001	<0.005
22:6ω3/22:5ω3	2.14	(1.14 - 2.97)	1.71	(0.77 - 3.64)	7 (11)	1 (2)	<0.005	<0.005
22:5ω6/20:4ω6	0.04	(0.02 - 0.05)	0.03	(0.02 - 0.06)	0 (0)	1 (2)	0.154	0.201
20:5ω3/22:6ω3	0.12	(0.06 - 0.20)	0.16	(0.07 - 0.33)	0 (0)	18 (30)[d]	<0.001	<0.001
22:5ω6/22:6ω3	0.13	(0.07 - 0.22)	0.16	(0.05 - 0.33)	3 (5)[c]	9 (15)	0.465	0.194
18:2ω6/18:3ω3	56.01	(36.03 - 82.31)	72.72	(28.48 - 142.78)	1 (2)	17 (28)	<0.001	<0.001

BMI correlated positively with RBC 20:0 and 20:3ω6 ($p<0.05$ for both). Duration of illness correlated negatively with LA ($p<0.005$; $r= -0.438$). Smokers had lower RBC 14:0 ($p<0.05$) and higher 24:1ω9 ($p<0.01$). Female smokers had lower 24:0 and 26:0 ($p<0.05$ for both) levels than male smokers. Alcohol users had higher 16:0, 20:0, ω9 (notably 18:1ω9), compared with non-alcohol users, whereas their 26:0, PUFA (notably ω3+ω6) and LCPω3+ω6 were lower ($p<0.05$ for all). The number of alcohol units consumed per day did not correlate with any fatty acid except for 22:5ω3 ($p=0.05$, $r=0.331$). Patients reaching the recommended fish-intake (i.e. $Q\geq4$; 11/52) had higher SAFA (notably 16:0), ω3, LCPω3 (notably EPA) and lower ω6, LCPω6 (notably AA and 22:4ω6) and PUFA/SAFA compared with patients who did not reach the recommended fish intake ($p<0.05$ for all). The index for fish-fat intake (Q) correlated ($p<0.005$ for all) with the ω3/ω6 ratio ($r=0.397$), LCPω3/LCPω6 ratio ($r=0.441$), EPA ($r=0.528$) and LCPω6 ($r= -0.414$).

In a logistic regression model ($p<0.05$) RBC PUFA below the P2.5 of the reference range proved best predicted in the total group by alcohol consumption (Wald $\chi^2 = 5.495$, $p<0.05$; odds ratio = 5.609, 95% CI 1.327 – 23.712) and duration of illness (Wald $\chi^2 = 4.246$, $p<0.05$; odds ratio = 0.822, 95% CI 0.683 – 0.988), when tested for alcohol consumption, duration of illness, age and gender. Linear regression showed the variance in RBC 20:3ω9 to be explained for 52% by ALA ($\beta= -0.298$, $p<0.005$), LA ($\beta= -0.505$, $p<0.001$) and DHA ($\beta= -0.369$, $p<0.001$) in the total group, when we tested for ALA, LA, 22:5ω6, DHA and SAFA. In females 51% of the variance in RBC 20:3ω9 could be explained by LA ($\beta= -0.727$, $p<0.001$), when testing for LA and ALA. In males LA ($\beta= -0.569$, $p<0.001$) and DHA ($\beta= -0.643$, $p<0.001$) explained 49% in RBC 20:3ω9 of the variance, when we tested for ALA, LA and DHA.

3.3. B-vitamins and homocysteine

Table 3 shows the B-vitamin status parameters of the patients, together with the percentage patients exhibiting values below and above the respective reference ranges. Between (sub)group comparisons were done with all patients and with the subgroup reporting no intake of vitamin supplements. B-vitamin status parameters were not related to BMI, DSM-IV classification, medication type and dose, and alcohol consumption. In our data set levels of homocysteine did not differ among the various age groups of male patients.

Table 3. *B-vitamin status parameters of patients with schizophrenia.*

	Reference values	Patients Median	(range)	Patients N<P2.5	n>P97.5	Patients vs. Controls P [a]	P [b]
Folate (nmol/L)	4.0 - 30.0	9	(3.4 - 38)	2 (3)	2 (3) [c]	0.121	0.079
Vitamin B_6 (nmol/L)	35 - 136	68	(26 - 382)	3 (5)	6 (10) [c]	0.587	0.317
Vitamin B_{12} (pmol/L)	170 - 700	211	(77 - 944)	17 (28)	1 (2)	<0.05	<0.001
Homocysteine (μmol/l)	4.7-14.6 [d]	11.6	(5.9 - 74.8)	0 (0)	17 (28)	<0.001	<0.001
	9.3 [e]			13 (21) [f]	48 (79)		

Data are for 61 patients. B-vitamin status parameters were measured in serum (folate, vitamin B_{12}), plasma (homocysteine) and whole blood (vitamin B_6). Data represent medians (range). The numbers (%) of patients with levels either below (n<P2.5) or above (n>P97.5) the reference ranges were assessed. Abnormal homocysteine was also assessed with the use of cut-off values at 9.3 and 14.6 μmol/L, which represent the P97.5 of B-vitamin optimized healthy adults and the P97.5 of these adults before B-vitamin optimization [44], respectively. Raw data for between-group comparisons derived from [44]. P<0.05 was considered significant for between group analyses. [a] Significance (p) for all patients (n=61) vs. controls (n=101); [b] Significance (p) for patients (n=57) vs. controls (n=79) reporting no intake of vitamin supplements; [c] Patients (n=2) reporting vitamin intake are included; [d] P97.5 derived from raw data [44] were used to evaluate individual patients; [e] Vitamin-optimized reference value [44]; [f] Patients (n=4) reporting intake of vitamins are included.

Most remarkable were the lower levels of vitamin B_{12} ($p<0.001$; 28% below P2.5) and the higher levels of homocysteine ($p<0.001$). Twenty-eight percent of patients, versus 2% of controls ($p<0.001$), had homocysteine levels above the P97.5 of the reference range; while 79% had values above the 'vitamin-optimized cut-off value'. A higher percentage of patients than controls had vitamin B_{12} levels below local reference values (28 vs. 3% below 170 pmol/L, $p<0.001$). Four patients, all males, aged 26-53 years, exhibited moderate hyperhomocysteinemia (range 57.5-74.8 μmol/L). None of these reported the intake of supplements. Vitamin B_6 levels were lower ($p<0.05$) in smoking than in non-smoking patients. Schizophrenic female smokers had lower homocysteine ($p<0.005$) than male smokers. Fish fat intake (Q) correlated positively with levels of folate ($p<0.01$, $r=0.371$) and vitamin B_{12} ($p<0.001$, $r=0.548$).

In a logistic regression model ($p<0.05$) a homocysteine above 14.6 μmol/L proved best predicted by male gender (Wald $\chi^2 = 4.774$, $p= 0.029$; odds ratio = 7.693, 95% CI 1.234 – 47.965) and absence of fish consumption (Wald $\chi^2 = 4.224$, $p= 0.039$; odds ratio = 0.1, 95% CI 0.011 – 0.894), when tested for gender, age, absence of fish consumption and smoking. The variance in plasma homocysteine could be explained for 47% by folate ($\beta= -0.611$, $p<0.001$) and ALA ($\beta= -0.317$, $p<0.005$) in the total group, when we tested for folate, vitamin B_{12}, vitamin B_6, fish fat intake, ALA and EPA. In females 45% of the homocysteine variance could be explained by

vitamin B_6 (β= -0.508, $p<0.01$) and vitamin B_{12} (β= -0.348, $p<0.05$), when we tested for folate, vitamin B_{12} and vitamin B_6. In males the homocysteine variance could be explained for 48% by folate (β= -0.701, $p<0.001$), testing for folate, vitamin B_{12} and vitamin B_6.

3.4. Dietary supplementation

None of the 5 patients with either biochemical EFA deficiency, moderate hyperhomocysteinemia or both was expected by their treating physicians to have poor diets. Four of them were available for supplementation with B-vitamins, and soybean and fish oils. The **Figure** shows the courses of their homocysteine and RBC 20:3ω9 during supplementation. The supplementation schemes were different for the 3 patients with hyperhomocysteinemia-only and the one with combined hyperhomocysteinemia and biochemical EFA deficiency. One of the 3 patients with hyperhomocysteinemia-only showed an immediate homocysteine decrease upon administration of the initial daily dosage of 800 μg folic acid, while the remaining 2 showed decreases upon the addition of other B-vitamins, including 8 mg vitamin B_6 and 4 μg vitamin B_{12}. The final homocysteine levels of these 2 patients were below 14.6 μmol/L (P97.5) and only one had a level below the more appropriate vitamin-optimized cut-off value of 9.3 μmol/L. The patient who did not reach a homocysteine below 14.6 μmol/L admitted to his clinician to be incompliant with respect to the intake of the B-vitamin supplement. His relatively low RBC folate level, as compared with the two others (data not shown) also suggested poor compliance, since RBC folate is a parameter of the folate status of the preceding weeks. The patient with combined hyperhomocysteinemia and biochemical EFA deficiency showed an initial increase of plasma homocysteine, which was probably due to our request to discontinue the reported infrequent intake of a multivitamin supplement. Addition of folic acid and other B-vitamins reduced homocysteine to a final level below 14.6 μmol/L but above 9.3 μmol/L.

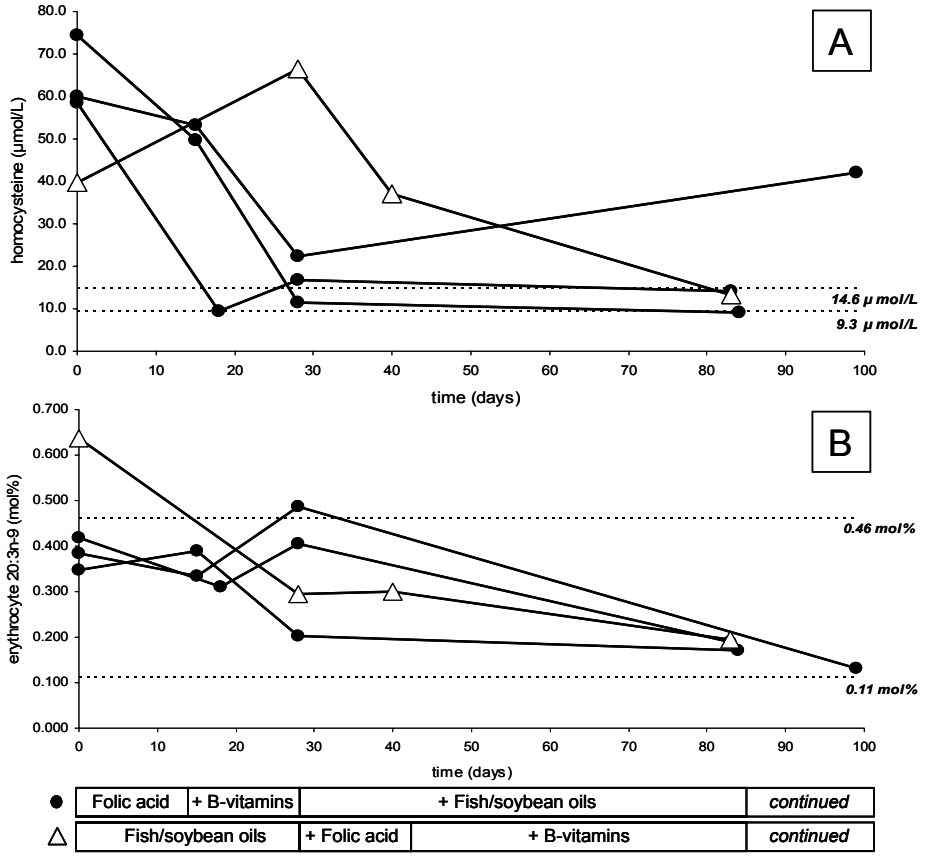

Figure. ***Courses of plasma homocysteine (A) and erythrocyte 20:3ω9 content (B) for 4 patients with schizophrenia during B-vitamin, soybean oil and fish oil supplementation.***

Selection of the 4 patients for supplementation was based on either moderate hyperhomocysteinemia (i.e. 31-100 µmol/L) or biochemical signs of essential fatty acid deficiency (erythrocyte 20:3ω9>0.46 mol%). Supplementation schemes (bottom) were different for the 3 patients with moderate hyperhomocysteinemia (•), and the one with combined hyperhomocysteinemia and essential fatty acid deficiency (△). Daily supplements were: Folic acid (800 µg folic acid), B-vitamins (8 mg vitamin B_6, 4 µg vitamin B_{12} and other B-vitamins), Fish oil (310 mg eicosapentaenoic acid (EPA; 20:5ω3) and 200 mg docosahexaenoic acid (DHA; 22:6ω3), and Soybean oil (15 mL soybean oil). The patient who exhibited an initial homocysteine decrease and a subsequent increase admitted to be incompliant with respect to B-vitamin intake.

Supplementation of the patient with combined hyperhomocysteinemia and biochemical EFA deficiency with soybean and fish oils rapidly reduced RBC 20:3ω9

to levels within the reference range. Also the 3 patients with hyperhomocysteinemia-only exhibited decreases of RBC 20:3ω9, although their changes occurred largely within the reference range. All patients seemed compliant with regard to the intake of the soybean oil and fish oil supplements, since all of them exhibited clear increases in their RBC LA, EPA and DHA (not shown).

4. Discussion

We assessed the frequency of low B-vitamin and EFA status, its determinants and its predictors in a group of patients with schizophrenia. Furthermore we were interested to see whether those with poor status responded biochemically to the appropriate dietary supplements.

Most remarkably we found that, as a group, the patients had high RBC SAFA and MUFA and low PUFA, both from the ω3 and ω6 series. Lower PUFA status seems in contrast to a recent study of Strassnig et al. [45] who reported increased fat, SAFA and PUFA intakes in schizophrenia. Their results were, however, not accompanied by laboratory data showing the actual PUFA status of their patients. RBC PUFA below the P2.5 of the reference range proved best predicted by alcohol consumption and duration of illness. Not unexpectedly, the index of biochemical EFA deficiency (i.e. RBC 20:3ω9) proved best explained by low RBC ALA, LA and DHA. Two patients exhibited biochemical EFA deficiency and 7 showed biochemical signs of ω3/DHA marginality only. Of these nine, at least 5 (i.e. ≥56%) reported not to eat fish at all as compared to 2% in the rest of the study population. The employed index for fish fat intake proved reliable, since there was also a positive relation between the calculated intake of fish oil fats and ω3LCP status. DSM-IV classification, duration of illness, gastrointestinal disturbances, BMI, smoking, alcohol drinking and medication were not related to the indices of EFA (20:3ω9) or B-vitamin (homocysteine) deficiencies.

With respect to B-vitamin status we found low vitamin B_{12} levels (28%) and high homocysteine (28%) in the group of patients not reporting to take vitamin supplements, and lower vitamin B_6 in smoking compared with non-smoking patients. A homocysteine above 14.6 μmol/L proved best predicted by male gender. Homocysteine variance proved best explained by folate and ALA in the total group. The percentage homocysteine variance explained by folate and vitamin B_{12} in our patient group was higher than corresponding data from a recent study in Israel [46]. The influence of ALA was unexpected, but in a subsequent analysis both folate and vitamin B_{12} proved positively related with our index of fish oil intake, suggesting that we were dealing with poor dietary habits in general and not e.g. diminished intake of certain food products. Four patients exhibited moderate hyperhomocysteinemia with plasma values ranging from 57.5-74.8 μmol/L. These

are exceptionally high values that in our experience are only rarely found in routine patient care. One of these patients also had biochemical EFA deficiency, which adds to the contention that some patients are subject to poor dietary habits in general. EFA deficiency [47] and hyperhomocysteinemia with homocysteine levels in Israeli schizophrenic patients up to 80 μmol/L have recently been reported [46]. It should be noted that our study was the first to show a low EFA status as derived from increased 20:3ω9. This fatty acid, also named Mead acid, is a functional parameter of the EFA status [38], and is rarely found to be increased in adults with undisturbed fat absorption consuming typically Western diets.

Importantly, none of the 5 patients with biochemical EFA deficiency, moderately increased homocysteine, or both, was suspected to have a poor diet, when we informed their clinicians of these outcomes. That diet is nevertheless at the basis of their abnormal indices of EFA and B-vitamin status became confirmed by the supplementation study that we performed in 4 of them. This study showed these indices to be easily correctable by supplemental B-vitamins, and soybean and fish oils, with folic acid probably being the most important determinant of hyperhomocysteinemia. However, 3 of the 4 patients did not reach the 9.3 μmol/L upper limit of the plasma homocysteine concentration, that we achieved by supplementation of healthy adults with a combination of folic acid, vitamin B_{12} and vitamin B_6 [38]. Prolonged supplementation or addition of betaine to the supplement might be of aid to reach this treatment goal, but compliance seems to be a more realistic subject of concern.

We conclude that, as a group, patients with schizophrenia have high RBC SAFA and MUFA, low PUFA and increased homocysteine. A subgroup of patients with schizophrenia has biochemical EFA deficiency, ω3/DHA marginality, moderate hyperhomocysteinemia, or combinations. This subgroup is not readily identified by their clinicians, while these conditions proved easily correctable by supplementation. Correction may be indicated in view of the possible relationship of poor EFA and B-vitamin status with their psychiatric symptoms, but also in view of the reduction of their high risk of cardiovascular disease. There is compelling, though not definite, evidence that reduction of homocysteine by B-vitamin supplementation (notably folic acid) and augmentation of ALA and ω3LCP status (by supplementation of soybean and fish oils) decreases risk of cardiovascular disease. Our study does not allow definite conclusions regarding risk factors for low PUFA and increased homocysteine that would allow their easy identification in clinical practice. Alcohol consumption and duration of illness seem to be risk factors for low PUFA status (i.e. <P2.5 of reference range), while male gender and absence of fish consumption seem to predict hyperhomocysteinemia (>P97.5). These factors and their predictive values should be investigated in larger studies. To

this end possible options for the identification of patients with poor diets are monitoring of dietary practices, or the more reliable, but also more costly, laboratory screening. Another possibility is supplementation of all patients without any monitoring or testing. The costs of either of these approaches and the anticipated compliance of intervention must be weighed against the potential clinical benefits with regard to the amelioration of psychiatric symptoms and the reduction of cardiovascular risk.

Acknowledgements

We thank Mrs. Ineke Kamminga for her valuable assistance in patient recruitment and Mr. Hans Kamphuis MD, for the physical examination of some of the participating patients. The patients and the clinical and research staff of the hospital are greatly acknowledged for their participation in this study.

References

1. Peet M, Shah S, Selvam K, Ramchand CN. Polyunsaturated fatty acid levels in red cell membranes of unmedicated schizophrenic patients. World J Biol Psychiatry 2004 Apr;5(2):92-9.
2. Fox H, Ross BM, Tocher D, Horrobin D, Glen I, St Clair D. Degradation of specific polyunsaturated fatty acids in red blood cells stored at -20 degrees C proceeds faster in patients with schizophrenia when compared with healthy controls. Prostaglandins Leukot Essent Fatty Acids 2003 Nov;69(5):291-7.
3. Horrobin DF. The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. Schizophr Res 1998 Apr 10;30(3):193-208.
4. Hibbeln JR, Makino KK, Martin CE, Dickerson F, Boronow J, Fenton WS. Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder. Biol Psychiatry 2003 Mar 1;53(5):431-41.
5. Reddy RD, Yao JK. Environmental factors and membrane polyunsaturated fatty acids in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2003 Dec;69(6):385-91.
6. Mellor JE, Laugharne JD, Peet M. Schizophrenic symptoms and dietary intake of n-3 fatty acids. Schizophr Res 1995 Dec;18(1):85-6.
7. Arvindakshan M, Sitasawad S, Debsikdar V, Ghate M, Evans D, Horrobin DF, et al. Essential polyunsaturated fatty acid and lipid peroxide levels in never-medicated and medicated schizophrenia patients. Biol Psychiatry 2003 Jan 1;53(1):56-64.
8. Khan MM, Evans DR, Gunna V, Scheffer RE, Parikh VV, Mahadik SP. Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. Schizophr Res 2002 Nov 1;58(1):1-10.
9. Hopper K, Wanderling J. Revisiting the developed versus developing country distinction in course and outcome in schizophrenia: results from ISoS, the WHO collaborative followup project. International Study of Schizophrenia. Schizophr Bull 2000;26(4):835-46.
10. Peet M. International variations in the outcome of schizophrenia and the prevalence of depression in relation to national dietary practices: an ecological analysis. Br J Psychiatry 2004 May;184:404-8.
11. Joy CB, Mumby-Croft R, Joy LA. Polyunsaturated fatty acid supplementation for schizophrenia. Cochrane Database Syst Rev 2003;(2):CD001257.
12. Arvindakshan M, Ghate M, Ranjekar PK, Evans DR, Mahadik SP. Supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. Schizophr Res 2003 Aug 1;62(3):195-204.
13. Peet M. Eicosapentaenoic acid in the treatment of schizophrenia and depression: rationale and preliminary double-blind clinical trial results. Prostaglandins Leukot Essent Fatty Acids 2003 Dec;69(6):477-85.
14. Applebaum J, Shimon H, Sela BA, Belmaker RH, Levine J. Homocysteine levels in newly admitted schizophrenic patients. J Psychiatr Res 2004 Jul;38(4):413-6.
15. Levine J, Stahl Z, Sela BA, Gavendo S, Ruderman V, Belmaker RH. Elevated homocysteine levels in young male patients with schizophrenia. Am J Psychiatry 2002 Oct;159(10):1790-2.
16. Muntjewerff JW, van der Put N, Eskes T, Ellenbroek B, Steegers E, Blom H, et al. Homocysteine metabolism and B-vitamins in schizophrenic patients: low plasma folate as a possible independent risk factor for schizophrenia. Psychiatry Res 2003 Nov 1;121(1):1-9.
17. Regland B, Johansson BV, Grenfeldt B, Hjelmgren LT, Medhus M. Homocysteinemia is a common feature of schizophrenia. J Neural Transm Gen Sect 1995;100(2):165-9.
18. Susser E, Brown AS, Klonowski E, Allen RH, Lindenbaum J. Schizophrenia and impaired homocysteine metabolism: a possible association. Biol Psychiatry 1998 Jul 15;44(2):141-3.
19. Goff DC, Bottiglieri T, Arning E, Shih V, Freudenreich O, Evins AE, et al. Folate, homocysteine, and negative symptoms in schizophrenia. Am J Psychiatry 2004 Sep;161(9):1705-8.
20. Virgos C, Martorell L, Simo JM, Valero J, Figuera L, Joven J, et al. Plasma homocysteine and the methylenetetrahydrofolate reductase C677T gene variant: lack of association with schizophrenia. Neuroreport 1999 Jul 13;10(10):2035-8.
21. Muntjewerff JW, Hoogendoorn ML, Kahn RS, Sinke RJ, Heijer MD, Kluijtmans LA, et al. Hyperhomocysteinemia, methylenetetrahydrofolate reductase 677TT genotype, and the risk for schizophrenia: A dutch population based case-control study. Am J Med Genet B Neuropsychiatr Genet 2005 May 5;135(1):69-72.
22. Luchsinger JA, Mayeux R. Dietary factors and Alzheimer's disease. Lancet Neurol 2004 Oct;3(10):579-87.
23. Mattson MP, Shea TB. Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci 2003 Mar;26(3):137-46.
24. da Costa KA, Gaffney CE, Fischer LM, Zeisel SH. Choline deficiency in mice and humans is associated with increased plasma homocysteine concentration after a methionine load. Am J Clin Nutr 2005 Feb;81(2):440-4.
25. Holm PI, Ueland PM, Vollset SE, Midttun O, Blom HJ, Keijzer MB, et al. Betaine and folate status as cooperative determinants of plasma homocysteine in humans. Arterioscler Thromb Vasc Biol 2005 Feb;25(2):379-85.
26. Steenge GR, Verhoef P, Katan MB. Betaine supplementation lowers plasma homocysteine in healthy men and women. J Nutr 2003 May;133(5):1291-5.

27. Davis CD, Uthus EO. DNA methylation, cancer susceptibility, and nutrient interactions. Exp Biol Med (Maywood) 2004 Nov;229(10):988-95.
28. Godfrey PS, Toone BK, Carney MW, Flynn TG, Bottiglieri T, Laundy M, et al. Enhancement of recovery from psychiatric illness by methylfolate. Lancet 1990 Aug 18;336(8712):392-5.
29. Ryan MC, Thakore JH. Physical consequences of schizophrenia and its treatment: the metabolic syndrome. Life Sci 2002 Jun 7;71(3):239-57.
30. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002 Nov 23;325(7374):1202.
31. Weiss N, Hilge R, Hoffmann U. Mild hyperhomocysteinemia: risk factor or just risk predictor for cardiovascular diseases? Vasa 2004 Nov;33(4):191-203.
32. Din JN, Newby DE, Flapan AD. Omega 3 fatty acids and cardiovascular disease--fishing for a natural treatment. BMJ 2004 Jan 3;328(7430):30-5.
33. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002 Nov 19;106(21):2747-57.
34. Leaf A, Kang JX, Xiao YF, Billman GE. Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils. Circulation 2003 Jun 3;107(21):2646-52.
35. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text-Revision. 4th ed. Washington: 1994.
36. Schrijver J, Speek AJ, Schreurs WH. Semi-automated fluorometric determination of pyridoxal-5'-phosphate (vitamin B6) in whole blood by high-performance liquid chromatography (HPLC). Int J Vitam Nutr Res 1981;51(3):216-22.
37. Muskiet FA, van Doormaal JJ, Martini IA, Wolthers BG, van der Slik W. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr 1983 Dec 9;278(2):231-44.
38. Fokkema MR, Smit EN, Martini IA, Woltil HA, Boersma ER, Muskiet FA. Assessment of essential fatty acid and omega3-fatty acid status by measurement of erythrocyte 20:3omega9 (Mead acid), 22:5omega6/20:4omega6 and 22:5omega6/22:6omega3. Prostaglandins Leukot Essent Fatty Acids 2002 Nov;67(5):345-56.
39. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004 Jan;50(1):3-32.
40. Hulshof KF, Ocke MC, Rossum CTv, Buurma-Rethans EJ, Brants HA, Drijvers JJ, et al. Results of the national food consumption survey 2003. Zeist: TNO Food; 2004. Report No.: 350030002/2004.
41. WHO. ATC/DDD Index 2004. WHO, Collaborating Centre for Drug Statistics Methodology . 2004.
42. Antipsychotics [computer program]. Washington, DC: American Psychiatric Press; 1998.
43. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA Dietary Guidelines: revision 2000: A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Stroke 2000 Nov;31(11):2751-66.
44. Fokkema MR, Weijer JM, Dijck-Brouwer DA, van Doormaal JJ, Muskiet FA. Influence of vitamin-optimized plasma homocysteine cutoff values on the prevalence of hyperhomocysteinemia in healthy adults. Clin Chem 2001 Jun;47(6):1001-7.
45. Strassnig M, Singh Brar J, Ganguli R. Dietary fatty acid and antioxidant intake in community-dwelling patients suffering from schizophrenia. Schizophr Res 2005 Jul 15;76(2-3):343-51.
46. Stahl Z, Belmaker RH, Friger M, Levine J. Nutritional and life style determinants of plasma homocysteine in schizophrenia patients. Eur Neuropsychopharmacol 2005 May;15(3):291-5.
47. Peet M, Laugharne JDE, Mellor J, Ramchand CN. Essential fatty acid deficiency In erythrocyte membranes from chronic schizophrenic patients, and the clinical effects of dietary supplementation. Prostaglandins Leukotrienes and Essential Fatty Acids 1996 Aug;55(1-2):71-5.